Chemosphere 197 (2018) 716–728

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

First report of metallic elements in loggerhead and leatherback turtle eggs from the Indian Ocean

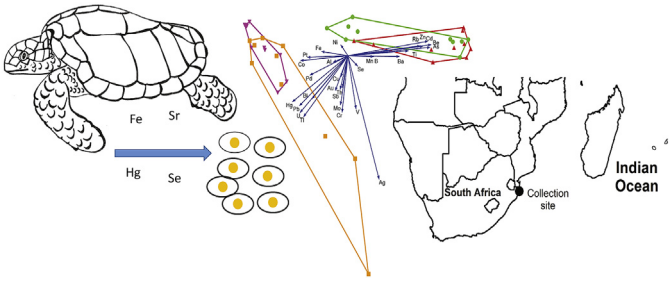
M. du Preez [a], R. Nel [b], H. Bouwman [a, *]

[a] Research Unit: Environmental Sciences and Management, North-West University, Potchefstroom, South Africa
[b] Department of Zoology and Institute for Coastal and Marine Research, Nelson Mandela University, Port Elizabeth, South Africa

HIGHLIGHTS

- First report of metals and metalloids in sea turtle eggs from the Indian Ocean.
- Loggerhead and leatherback eggs collected from the South African coast, and analysed.
- Life-history differences accounted for much of the differences in concentrations.
- Cu and Sr exceeded levels of concern, but no indication of reproductive failure.
- Eggshells cannot be used as proxy for egg contents - no elemental congruence.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 22 June 2017
Received in revised form
20 January 2018
Accepted 22 January 2018
Available online 3 February 2018

Handling Editor: Martine Leermakers

Keywords:
Reptile
Heavy metal
Toxic reference value
Endocrine disruption
Climate change
Deme discrimination

ABSTRACT

Bio-monitoring of pollutants in long-lived animals such as sea turtles is an important tool in ecotoxicology. We present the first report on metallic elements in sea turtle eggs from the Indian Ocean. Eggs of the leatherback and loggerhead turtle that breed on the Indian Ocean coast of South Africa were analysed for 30 elements. The eggshells and egg contents of the loggerhead turtle, the smaller of the two species, had higher or significantly higher concentrations than leatherbacks, except for strontium - the reason is unknown. Elemental concentrations in eggshells and contents were the same or lower compared with other studies. The differences in concentrations in the egg contents and eggshells between the two species are likely due to different trophic levels, migration patterns, life histories, age, and growth, as well as differences in pollution sources and the uptake, retention and elimination characteristics of the different elements by the different species. We found no congruence between patterns in eggshells and corresponding egg contents, for both species. However, eggshells and egg contents showed congruence between species. The lack of congruence between eggshells and contents within each species precludes using eggshell concentrations as a proxy for egg content concentrations. Copper, strontium, and selenium occurred at concentrations higher than available toxic reverence values. Further research is warranted, including the analyses of POPs, as well as possible deme discrimination based on compositional pattern differences. Turtles serve as 'active samplers' returning to the same location to breed—something that is not practical with marine mammals or elasmobranchs.

© 2018 Elsevier Ltd. All rights reserved.

* Corresponding author.
E-mail address: henk.bouwman@nwu.ac.za (H. Bouwman).

https://doi.org/10.1016/j.chemosphere.2018.01.106
0045-6535/© 2018 Elsevier Ltd. All rights reserved.

1. Introduction

Metallic elements are present naturally in the environment but concentrations elevated above background can occur due to anthropogenic activities such as mining, agriculture, urban runoff, and the combustion of fossil fuels (Burger, 2002; Greenfield et al., 2011; Wepener and Degger, 2012).

Certain metals such as lead, cadmium, and mercury have no known biological functions and have adverse negative effects on biota even at low concentrations (Järup, 2013; Nordberg et al., 2015). On the other hand, some metals and metalloids, such as cobalt, molybdenum, iron, zinc, manganese, selenium, arsenic, and copper, are essential to the physiology and development of animals, but can become toxic at elevated levels (Nordberg et al., 2015). Metals and metalloids are absorbed, inhaled, and ingested trough water, air, food, and other sources by humans and biota. Metals and metalloids, such as lead, cadmium, selenium, arsenic, and mercury can also bio-accumulate (Nordberg et al., 2015).

Monitoring of concentrations of metals and metalloids in biota and the environment is important to identify situations where they might cause biological harm. To this effect, bio-monitoring of pollutants in long-lived species is an important tool; it provides a baseline measurement for further studies into the health of a population, and increases knowledge about the levels of pollution in the areas where these animals live (Burger, 2002; Cortés-Gómez et al., 2014; Finlayson et al., 2016). It also allows risk to be assessed, especially when compared with known levels of concern and with datasets from around the world (Lam et al., 2006a,b; van de Merwe et al., 2009, 2010; Perrault et al., 2011; Villa et al., 2017).

Species differ in the rate and extent of uptake of different metals and metalloids depending on various factors that include habitat, lifespan, food preference, amounts of food, and the trophic level of the animal (Alava et al., 2006; Sola and Prat, 2006; Guirlet et al., 2008). In addition, the body burden of metals or metalloids that are taken up by an organism depends on its bio-availability (Ng et al., 2013). Marine turtles are presumed good bio-indicators of metals and metalloids in the marine environment as they have a long life expectancy once adult, and thus are exposed to and can take-up and/or bio-accumulate a variety of different substances (D'Ilio et al., 2011; Finlayson et al., 2016).

The loggerhead (*Caretta caretta*) and leatherback turtle (*Dermochelys coriacea*) in the Indian Ocean are under threat; the south-western Indian Ocean population of loggerheads is considered Near Threatened by the International Union for Conservation of Nature (IUCN) (IUCN, 2015); whereas the leatherback population in the same region is considered Critically Endangered (IUCN, 2013). Threats to both species include fisheries bycatch, harvesting, pollution, and climate change (IUCN, 2013, 2015). Both species nest on the Indian Ocean coast of South Africa and southern Mozambique.

Loggerhead turtles, the smaller of the two species, have an average curved carapace length of 86.4 cm and a hatching success of 78% in South Africa (Hughes, 1974). Loggerheads are more abundant than leatherbacks (Nel et al., 2013). IUCN (2015) does not provide population numbers, but estimated that 200 000 clutches are laid globally per annum, of which 4700 by the South West Indian Ocean population. Adult loggerhead females remain in their feeding areas until their next nesting migration; their migratory routes can vary—although most tend to stay near the coast for orientation, some do travel far out to sea (Papi et al., 1997; Luschi and Casale, 2003). Loggerheads mainly consume crustaceans and molluscs while in coastal habitats and jellyfish during pelagic journeys (Hughes, 1974; Stoneburner et al., 1980; Sakai et al., 2000).

Leatherbacks are the largest of all turtles with adults reaching a curved carapace length of 140–200 cm (Eckert et al., 2012; Hughes, 1974). One large adult female has been measured at 203 cm (Hughes, *pers. com*) Adults can weigh between 200 and 700 kg (Guirlet et al., 2008), and reach ages between 14 and at least 30 years (Tucek et al., 2014). The leatherback has an emerging success of 68.9% in South Africa (Hughes, 1974). IUCN (2013) does not provide population estimates nor clutches laid per year. The Indian Ocean population is however, decreasing. The migration pattern of the leatherbacks is wider than the loggerheads; they tend to move along the ocean currents and travel far out to sea, but some also remain close to the eastern African coast (Robinson et al., 2016). Leatherbacks feed exclusively on jellyfish, a lower trophic level than the loggerheads (Mrosovsky et al., 2009; Eckert et al., 2012; Robinson et al., 2016).

Because of the extensive migration distances, turtles in the Indian Ocean as elsewhere are exposed to a variety of environmental toxicants. Turtles consume food and water that might contain toxicants, including metals and metalloids (Bels et al., 1998; Talavera-Saenz et al., 2007; Guirlet et al., 2008; Perrault, 2014). Arsenic and cadmium can influence animal growth rate, foraging, mortality, and hatching success and can also influence their reproduction later on in life (Jakimska et al., 2011). Reptiles in early life stages are more sensitive to toxic effects of chemicals and metals compared with adults (Hopkins et al., 1999; Brasfield et al., 2004). Maternal blood Hg concentrations were not correlated with hatching success. It is thus important to monitor metals and metalloids in hatchlings or eggs to indicate if maternal transfer of pollutants poses a threat to the animal's early live stages.

There is remarkably little known about concentrations of metals and metalloids in marine turtle eggs. Much more information is available on metals and metalloids in blood, liver, and kidneys (Aguirre et al., 1994; Storelli and Marcotrigiano, 2003; Gardner et al., 2006). To the best of our knowledge, no previous work has been published on metallic elements in sea turtles in the Indian Ocean, the third largest ocean globally. Studies have been done on metallic elements in loggerhead and leatherback turtle eggs in other oceans though (Sakai et al., 1995; Godly et al., 1999; Guirlet et al., 2008; Roe et al., 2011). We report here a study on the concentrations and potential associated risks of metalloids and metals in sea turtle eggs from the Indian Ocean beaches of South Africa. We compared the differences between the two species due to their different trophic levels and feeding areas. We investigate whether eggshells can be used as proxy for egg contents, thereby reducing the impact on hatching. We also investigated whether the eggshells of the more abundant loggerheads can be used to infer patterns and concentrations in the scarcer leatherbacks.

2. Materials and methods

2.1. Study site and sample collection

The sea turtle eggs were collected in the iSimangaliso Wetland Park, World Heritage Site on the KwaZulu-Natal coast of South Africa (Fig. 1), between 1 January 2015 and 2 February 2015 (under DEA Permits RES2014/64 & RES2015/67, and research agreement with iSimangaliso Authority). The park stretches from the Mozambique border in the north at Kosi Bay, to about 180 km south to the St. Lucia lighthouse.

Due to other ongoing projects, the collection procedures differed between the two species. Nesting female leatherbacks were located during night-time beach patrols. Once located, each female was carefully observed from a distance until she had dug a body pit and egg chamber and started laying eggs. We removed two eggs from each clutch after about the 20th egg was laid to disturb the female as little as possible. Loggerhead eggs were collected

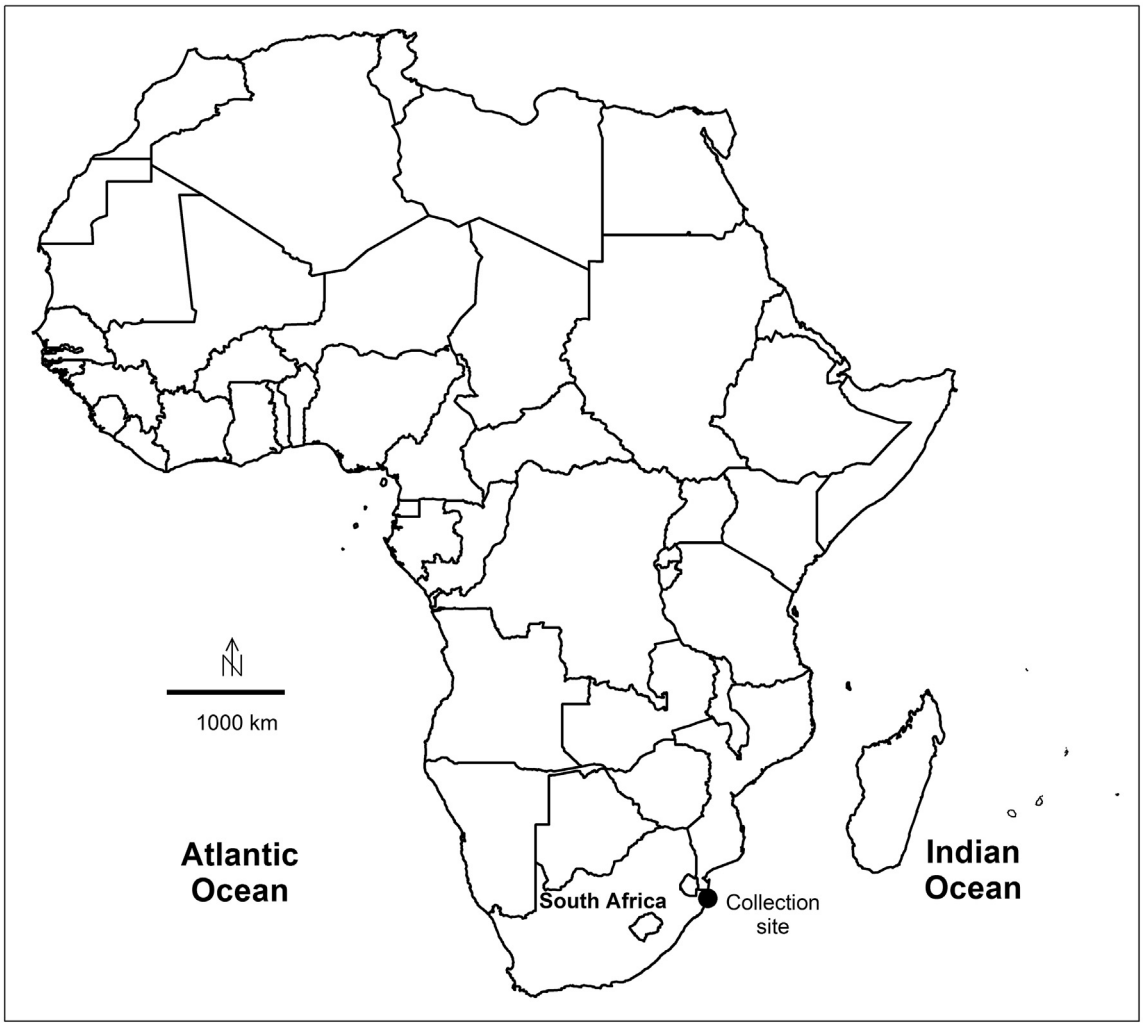


Fig. 1. Map indicating the sampling site in South Africa.

during a survey to calculate hatching success of nests; an emerging nest would be spotted by tracks left by hatchlings during the night. The nest would be marked and left for three or four days to ensure all hatchlings capable of emerging from the sand have left the nest. After the third or fourth day, the nest would be excavated by hand and the sand placed aside. All the egg shells, dead hatchlings, and undeveloped eggs were counted. Eggs where the embryo ceased to develop (due to unknown natural causes) were collected. The collected eggs (10 per species) were sealed in Ziploc bags in the field and stored at −20 °C until analyses.

2.2. Sample preparation

In the laboratory, the frozen eggs were carefully rinsed with deionized water to remove any sand particles. Eggs were weighed, measured and then separated into eggshell and egg contents (yolk and albumin). The mass of the wet content was determined before being ultrasonically homogenised, and a sub-sample placed in high-density polyethylene bottles. The sub-sample was freeze-dried (for 48 h at −80 °C and 0.133 Pa) and then powdered. The eggshells were air-dried for seven days. Half of each eggshell was crushed into a powder using a mortar and pestle and placed in polypropylene tubes with polyethylene screw caps. Any equipment that came in contact with sample were thoroughly rinsed and dried between samples.

The laboratory (Eco-Analytica at the North-West University) analysed the samples for 30 elements together with a certified standard reference material (SRM, 1944 – mussel tissue). Two grams of dried sample was acid- and heat-digested using 65% nitric acid, 50% hydrogen peroxide, and 33% hydrochloric acid (all reagent grade; Associated Chemical Enterprises) according to the procedures described in EPA3050b for ICPMS analyses. The digested solutions were analysed using an inductively-coupled-plasma mass-spectrometer (Agilent 7500 CE ICP-MS) fitted with a Micromist-type nebulizer and standard quartz chamber. The instrument is optimized using a solution containing Li, Y, Ce, and Tl (1 μg/L) for standard low-oxide/low interference levels (≤1.5%) while maintaining high sensitivity across the mass range. ULTRASPEC certified mixed multi-element stock standard solutions (De Bruyn Spectroscopic Solutions) containing all the elements of interest is used for calibration. Detection limits for each element are calculated from the calibration curves with each calibration run.

Concentrations were within 25% of certified values (Table S1). All of the elements in all samples had quantifiable concentrations above detection limits. Concentrations are expressed in mg/kg wet mass (wm) for egg contents, and mg/kg dry mass (dm) for shells.

2.3. Statistical analyses

Summary statistics and comparisons were done using GraphPad Prism 5.04 (www.graphpad.com). We used linear regression of all elemental concentrations between eggshells and contents of the same species with untransformed data (60 regressions), to determine which elemental concentrations in the contents can be predicted from the shell. In a few cases, where the data were not normally distributed, log-transformed data were used. We also compared elemental concentrations (un-transformed) between species using non-parametric, un-paired, two-tailed, Mann-Whitney t-tests.

We performed additional data analyses to compare the variation of the concentrations per sample type (contents or shell). Standard deviations (SDs) are not appropriate because of the many orders of differences of the concentrations precludes direct comparisons (Table 1, and supplemental Table S1). The percentage coefficient of variation (%CV) is a measure of relative variability (SD divided by the mean, and expressed either as a fraction between 0 and 1, or as a percentage) and independent of measurement unit, therefore without dimension. Using %CV allows direct comparisons of the variations in concentrations that differ over multiple orders of magnitude.

Since the eggshell and contents of the same egg is essentially paired, we used Wilcoxon matched-pairs, signed rank tests (non-parametric) to compare untransformed %CV values. The effectiveness of pairing is calculated using the Pearson correlation coefficient. The smaller the p-value, the more effective the pairing between the values of the concentrations between egg and contents for the whole dataset. Comparing the data between species cannot be paired, and hence we used the non-parametric Mann-Whitney test that is sensitive to differences of the medians.

To investigate the proportional compositions ('fingerprints') of the egg contents and eggshells, we used multivariate comparisons, using PC-Ord version 6.20 (MjM Software Design, www.pcord.com). Concentration data were relativized per sample to compensate for the large differences in concentrations. We used non-metric multidimensional scaling (NMS), with Sørensen as distance measure. A maximum of four axes were allowed, 200 maximum number of iterations, random starting conditions, 50 runs with real data, and 50 runs with randomised data, to stress-test the final ordination. A final stress value of less than five provides an excellent representation of the relationships between samples (McCune and Grace, 2002).

Multi-response permutation procedures (MRPP) were used to compare elemental profiles between eggshells and contents within each species. Concentrations were relativized per egg. The distance measure used was Euclidian. A *T*-statistic was calculated describing the separation between the groups – the more negative, the stronger the separation between the groups. A chance-corrected within-group agreement (*A*) was also calculated ($A = 1$, when all values are identical between groups; $A = 0$, when heterogeneity within groups equals expectation by chance), as well as the probability of a smaller or equal difference in profile (p value).

3. Results

3.1. Analytical results

The mean metal and metalloid concentrations of seventeen elements in egg contents and eggshells of the two turtle species, as well as comparable data from related literature, are presented in Table 1. The statistical summary for our study is available in Table S1 (Supplemental materials). Linear regressions of concentrations in leatherback egg contents and eggshell were only significant for Se ($p = 0.0107$, $r^2 = 0.58$), Sb ($p = 0.0170$, $r^2 = 0.53$), and Ba ($p = 0.0330$, $r^2 = 0.45$) — all three had positive relationships. For loggerheads, a positive relationship was found for Zn ($p = 0.0195$, $r^2 = 0.51$), and a negative relationship for Tl ($p = 0.033$, $r^2 = 0.68$). The graphs are shown in Supplemental materials, Fig. S1.

Of the 30 elements tested, loggerhead egg contents had significantly (un-paired, two-tailed, Mann-Whitney t-tests, $p < 0.05$) higher concentrations of Ti, Ni, Cu, Zn, Ag, Pt, Tl, and Bi than leatherback egg contents. Only Be was significantly higher in leatherback egg contents compared with loggerhead egg contents. The other elements were not significantly different.

Loggerhead eggshells had significantly higher concentrations of Be, Ti, V, Cr, Mn, Cu, Zn, As, Mo, Sb, Au, Hg, Tl, Pb, Bi, Th, and U than leatherback eggshells. Leatherback eggshells had significantly more Be and Sr than loggerhead eggshells. The other elements were not significantly different.

The mean and median %CV of the eggshells were higher than their respective egg contents in both species (Table 2), but was only significantly so for loggerheads ($p < 0.0001$). Pairing of matched samples were not effective ($p > 0.05$). The element Tl had the smallest %CV in egg contents in both species. Silver (Ag) had the largest %CV for eggshells of the Loggerhead, and eggshell and egg contents of the Leatherback.

3.2. Multivariate analyses

MRPP analyses of turtle eggshell and egg content concentrations showed significant differences for both species (Table 2). There was a much stronger separation between egg contents and eggshell for leatherback ($T = -12.69$, $A = 0.62$, $p < 0.0001$) than for loggerhead turtle eggs ($T = -7.54$, $A = 0.24$, $p < 0.0001$).

NMS ordinations comparing the relative concentrations of metals and metalloids in eggshells and egg content of leatherback and loggerhead turtles are presented in Fig. 2a and b, respectively. Leatherback egg content and eggshell profiles, as represented by their respective convex hulls, ordinated distinctly apart (Fig. 2a). Loggerhead eggshell and egg contents had profiles that were distinct but overlapped to a small extent; only two egg content samples ordinated within the convex hull of the eggshell profile (Fig. 2b). For both species, eggshells were characterised by relatively higher relative proportions of As, Be, Zn, Cd, Ti, and Ba.

Egg contents were characterised by relatively higher proportions of U, Hg, Tl, Ti, Rb, Pb, and Co. Loggerhead eggshells had relatively more Ag and Pt compared with leatherback shells, while it occurred in higher proportions in loggerhead egg contents than in leatherbacks. Vanadium was more prevalent in loggerhead shells, but occurred in higher proportions in leatherback egg contents. Elements with shorter vectors (Fig. 2a and b) in both species were Cu, Fe, and Se, contributing little to the separations.

NMS ordination of the matrixes of both species combined (Fig. 3a) shows a strong overlap of the egg contents of both species, but distinct separation of the eggshells from both egg contents and each other. Strontium was strongly associated with leatherback shells, ordinating opposite Ag. Removing Sr from the ordination calculations produced Fig. 3b. Titanium, As, Cd, Be, Zn, Rb, and Ba were strongly associated with contents of both eggs, while Tl, U, Pb, and Hg were strongly associated with loggerhead shells.

4. Discussion

Inter- and intra-clutch variations in elemental concentrations might confound the interpretation of analytical data. Sakai et al. (1995) investigated these confounding factors on three captive green turtles, and found no effect of egg-laying order within a clutch, nor intra-clutch variations from the same female, for

Table 1
Mean concentrations (μg/kg dm) of selected elements in loggerhead and leatherback turtle egg contents and eggshells from South Africa, as well as comparable data from other studies.

Species	Matrix	Year	Sample	Location	Al	Cu	Hg	Pb	Cr	Ni	Co	Cd	Zn	Se	As	Mn	Fe	V	Pt	Au	Ag	Reference
Hawksbill turtle	Content	2011	Dry	Iran		2.3	0.007	3.1		2.26		0.42	34			2.1						(Ehsanpour et al., 2014)
Flatback turtles	Content	2006	Wet	Australia		0.41	<0.05	<0.05	0.17	<0.05	<0.05	<0.05	10	0.3	0.67	0.17						(Ikonomopoulou et al., 2011)
Olive Ridley turtles	Content	2008	Dry	Mexico		2.2		0.74		3.3		0.24	72									(Paez-Osuna et al., 2010)
Olive Ridley turtles	Content	2008	Wet	Panama		0.6	0.009	0.004				0.07	16		0.12	0.35	11					(Ross et al., 2016)
Olive Ridley turtles	Content	2013	Wet	Panama		0.5	0.006	0.003				0.09	14		0.12	0.31	12					(Ross et al., 2016)
Green turtles	Content	2008	Wet	Oman		11			1.4		0.26	0.31										(Bicho et al., 2008)
Green turtles	Content	1994	Dry	Cyprus				0.66				0.33										(Ehsanpour et al., 2014)
Green turtles	Content	2006	Wet	Malaysia		0.5		0.031				0.009	15	0.46	0.097							(van de Merwe et al., 2009)
Green turtles	Content	2005	Wet	Malaysia		0.7		0	0.48			0	18	0.45	0	0.27						(Ikonomopoulou et al., 2009)
Green turtles	Content	2001	Wet	China	0	0.3	0.015	0.049	9.4	0.19	0.0030	0	45	3.5	2.5	0.27	45	0.22			0.027	(Lam et al., 2006a,b)
Leatherback Turtle	Content	2002	Wet	Costa Rica		26				1.9		1.6	14			0.9	40					(Reo et al., 2011)
Leatherback Turtle	Content	2008	Wet	Florida USA			0.048															(Perrault et al., 2011)
Leatherback Turtle	Content	2006	Wet	French Guiana		0.63	0.012	0.036				0.024	14	1.4								(Guirlet et al., 2008)
Leatherback Turtle	**Content**	**2015**	**Wet**	**South Africa**	**7.5**	**1.8**	**0.0005**	**0.0061**	**3**	**0.35**	**0.043**	**0.046**	**38**	**2.5**	**0.88**	**0.97**	**57**	**0.26**	**0.00036**	**0.18**	**0.17**	**This Study**
Loggerhead turtle	**Content**	**2015**	**Wet**	**South Africa**	**18**	**4.6**	**0.0006**	**0.00093**	**3.5**	**1.9**	**0.1**	**0.041**	**48**	**2.8**	**2.3**	**1.9**	**110**	**0.32**	**0.00041**	**0.2**	**0.35**	**This Stusy**
Loggerhead turtle	Content	1980	Wet	Florida USA	6.3	6.0	1.4	2.2	1.1		0.048	0.11	77				72					(Stoneburner et al., 1980)
Loggerhead turtle	Content	1980	Wet	Georgia USA	3.8	5.0	1.4	1.1	1.2	0.25	0.038	0.19	74				71					(Stoneburner et al., 1980)
Loggerhead turtle	Content	1980	Wet	North Carolina USA	4.1	5.4	0.63	1.8	1.0	2.3	0.072	0.04	79				73					(Stoneburner et al., 1980)
Loggerhead turtle	Content	1994	Dry	Cyprus			0.19	0.19				0.23										(Godley et al., 1999)
Loggerhead turtle	Content	1990	Wet	Japan		1.1	0.0055					0.013	15			0.52	12					(Sakia et al., 1995)
Leatherback Turtle	**Shell**	**2015**	**Dry**	**South Africa**	**26**	**8.6**	**0.16**	**0.25**	**11**	**0.72**	**0.54**	**0.00058**	**5.8**	**11**	**0.095**	**0.73**	**375**	**0.47**	**0.00036**	**0.72**	**0.33**	**This study**
Loggerhead turtle	**Shell**	**2015**	**Dry**	**South Africa**	**44**	**16**	**0.5**	**0.77**	**38**	**1**	**0.64**	**0.0011**	**16**	**5.8**	**0.83**	**2.0**	**390**	**2.4**	**0.013**	**2.1**	**2.3**	**This study**
Loggerhead turtle	Shell	2001	Dry	Turkey		13						1.6	12									(Kaska and Furness, 2001)
Loggerhead turtle	Shell	1990	Dry	Japan		5.5	0.004						2.2			0.68	11					(Sakia et al., 1995)
Green turtles	Shell	2001	Dry	Turkey		2.5		0.04	0.56	3.7		0.58					49					(Celik et al., 2006)
Olive Ridley turtles	Shell	1996	Dry	India		7.6				13		1.3	13									(Sahoo et al., 1996)
Olive Ridley turtles	Shell	2010	Dry	mexico		7.5				49		0.47	12									(Paez-Osuna et al., 2010)
Green turtles	Shell	2001	Dry	China	0	1.3	0.0061	0.11	0.47	12	3.3	0.016	1.2	2.5	0.22	0.27	2.3	0.079			0.034	(Lam et al., 2006a,b)
Hawksbill turtle	Shell	2011	Dry	Iran		6.6	0.002	4.2				0.36	5.6									(Ehsanpour et al., 2014)

Rows in bold indicate results of the current study.

Table 2
Summary statistics and the results of the *t*-test and multi-response permutation procedures (MRPP) of the percentage coefficients of variation (%CVs) of the concentrations in turtle egg contents and shells.

	Matrix	Mean	Median	SD	Min	Element	Max	Element	T-tests			MRPP			MRPP no Sr		
									Wil	M-W	Eff pairing	*T*	*A*	p	*T*	*A*	p
Leatherback	Shell	61.8	48.5	48.5	19.7	Rb	227	Ag	0.0724		0.1904	−13	0.6	<0.0001	−12	0.6	<0.0001
	Contents	52.2	27.4	56.9	16.8	Tl	260	Ag									
Loggerhead	Shell	79.0	67.0	48.1	34.4	Ti	255	Ag	<0.0001		0.2356	−7.5	0.2	<0.0001	−6.9	0.25	0.0004
	Contents	43.4	31.6	36.3	1.36	Tl	129	Ni									
Shell LH vs LB										0.0078							
Contents LH vs LB										0.6099							

elemental concentrations in yolk, except for Hg. For Hg at low concentrations (between 0.001 and 0.004 μg/g wm), the concentrations approximately halved between clutches 1 and 4. Therefore, the effects of egg-laying order and intra-clutch variation can be safely ignored, except for Hg. For Hg therefore, the highest concentration in eggs from nests of which the clutch sequence is unknown might be doubled as a worst-case scenario and then compared with other data and effect concentrations. Allochthonic and/or autochthonic uptake of elements from sand will be dealt with below.

4.1. Elemental concentrations

There were major differences in elemental concentrations within and between the two species (Tables 1 and S1, and Section 3.1). Loggerheads had significantly higher concentrations of eight elements in their egg contents and 17 elements in their eggshells than leatherbacks. Leatherback eggshells however, had higher concentrations of Sr and Be compared with loggerheads.

Compared with other studies (Table 1), loggerhead egg contents from South Africa had the highest Al, Mn, Fe, V, and Ag concentrations, although very little data is available for some elements. For eggshells, Cu, Hg, Pb, Cr, Zn, and Fe occurred at higher concentrations in loggerheads than elsewhere (Table 1). Both species had low concentrations of Pb and Hg in their egg contents compared with other studies (Table 1). The concentrations of Ni, Cu and Cd were higher in the egg content of leatherbacks from Costa Rica than from South Africa (Table 1).

4.2. Reasons for differences between species

The differences between our results and those of others (Table 1), although not major, are likely ascribable to a combination of geographic separation of the South African population ranges (the southern Indian and Atlantic oceans) with different elemental background concentrations, differences in life histories, gender, diets, age, growth, as well as differences in pollution sources and the uptake, retention and elimination characteristics of the different elements in different species (see also Godly et al., 1999; Perrault et al., 2011). Leatherbacks tend to stay further away from coastal areas that are more polluted by human activity. Their remote journeys may explain, in part, the generally lower levels of metals and metalloids (Papi et al., 1997; Luschi et al., 2006; Robinson et al., 2016). Loggerheads, which are more neritic, spend more time in polluted coastal regions.

Richards Bay, located 200 km south of the major rookeries of the present study, is a major export harbour for minerals, and a major site for dune mining of minerals from sand. Mussels have been assessed for metal pollution with a variable but unclear concentration trends over the decades since the mine was opened (Greenfield et al., 2011). Loggerheads spend more time along the coasts and are therefore likely to encounter water and food with elevated elemental concentrations.

Leatherbacks are however, also much larger than loggerheads. The relationship between body size and body burden of organic and inorganic compounds (body burden allometry) remains complex and difficult. Studies suggest that larger animals have lower burdens than smaller ones, including effects of dilution by growth (Newman and Heagler, 1991). Loggerhead eggs from the Mediterranean had higher concentrations of Pb and Cd than green turtles (Godly et al., 1999), despite their overlapping feeding grounds.

However, allometric confounding may be superseded by differences in diet; loggerheads largely feed on molluscs and crustaceans in coastal habitats and jellyfish during pelagic journeys, while leatherbacks feed almost exclusively on jellyfish (Eckert et al., 2012; Mrosovsky et al., 2009; Robinson et al., 2016). Loggerhead turtles also ingest more marine debris due to their coastal nature where higher concentrations of marine debris may be expected (Godly et al., 1999). Marine debris is likely to contain metals and metalloids such as Pb, Sr, Sb, and Fe (Hughes, 1996; Golloch and Siegmund, 1997; Nicolau et al., 2015) that may be taken up by the turtles after ingestion.

4.3. Autochthonic uptake by the shells?

There may also be an effect of autochthonic uptake by eggshells from the sands explaining the elemental concentration differences in eggshell and egg contents. Loggerhead eggs considered here were already two months in the sand (because unhatched eggs were used), while the leatherback eggs were freshly harvested. Given the soft and leathery nature of the shells, it may be that mobile elements migrated into and out of the eggshell and egg contents (Marco et al., 2004; Guirlet et al., 2008), although protective effects from eggshell and membrane have been reported (Nagle et al., 2001). Autochthonic uptake may have occurred, although the differences in species (diet and migratory ranges as discussed above) should be kept in mind. If there were uptake, it should be evident at least in the eggshells. Table 1 and supplementary data shows that the older eggs of loggerhead turtles had significantly higher concentrations of eight elements in their contents, and seventeen elements in their shells, compared with the fresher leatherback turtle eggs. Autochthonic uptake therefore cannot be excluded based on our data and may need further investigation.

4.4. Strontium

One of the main differences between egg content and eggshell was the significantly higher mean Sr and Be concentrations in leatherback eggshells when compared with loggerheads. Beryllium (also an alkaline earth metal as are Ca and Sr) concentration differences in the shells, although significantly different between the two species (Table 1), may have been due to variations at very low concentrations (0.0001 vs 0.0002 mg/kg dm). On the other hand,

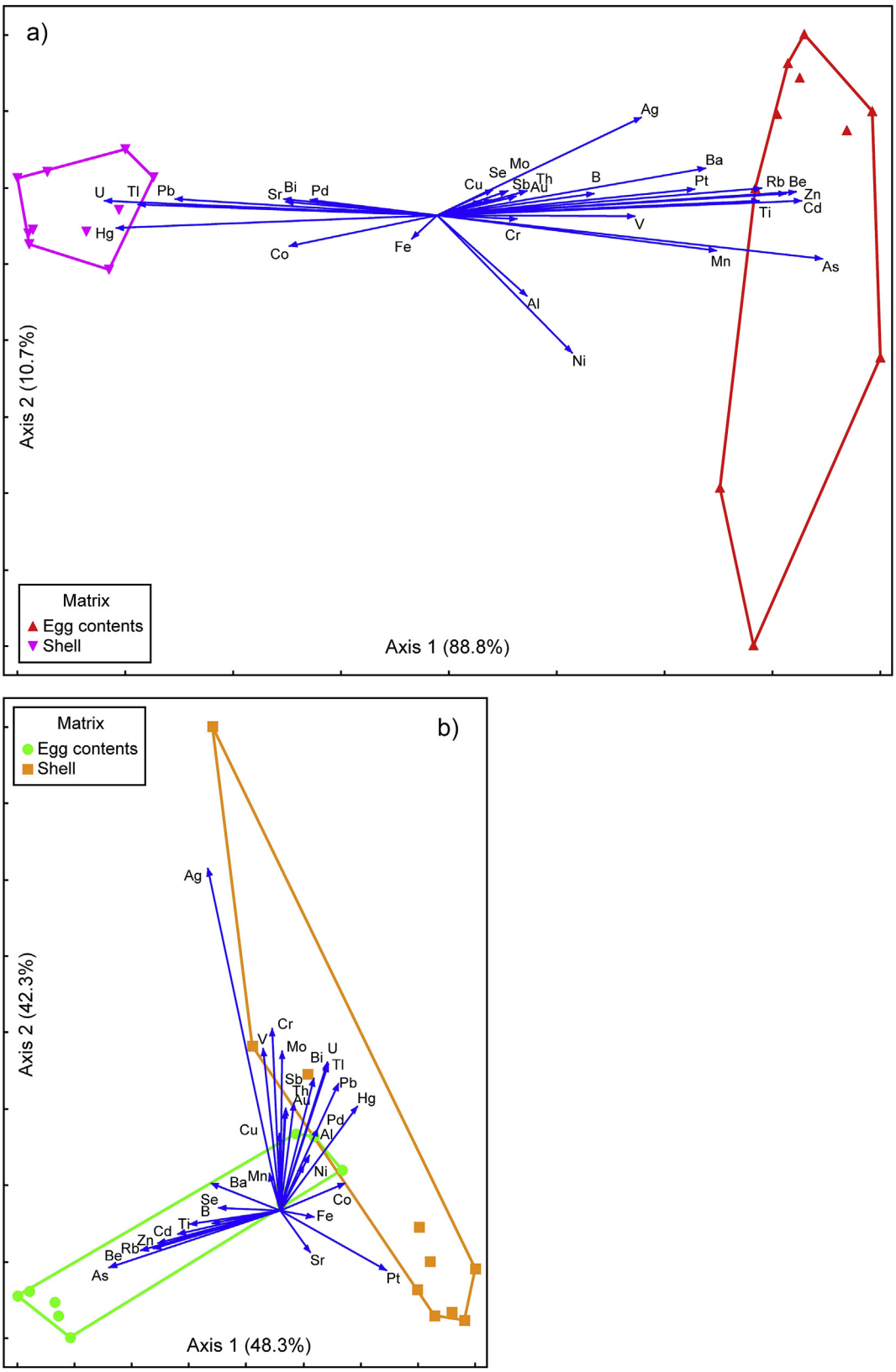


Fig. 2. a) Non-metric multidimensional scaling of relativized metal and metalloid concentrations in leatherback turtle eggshells and egg contents. b) Non-metric multidimensional scaling of relativized metal and metalloid concentrations in loggerhead turtle eggshells and egg contents.

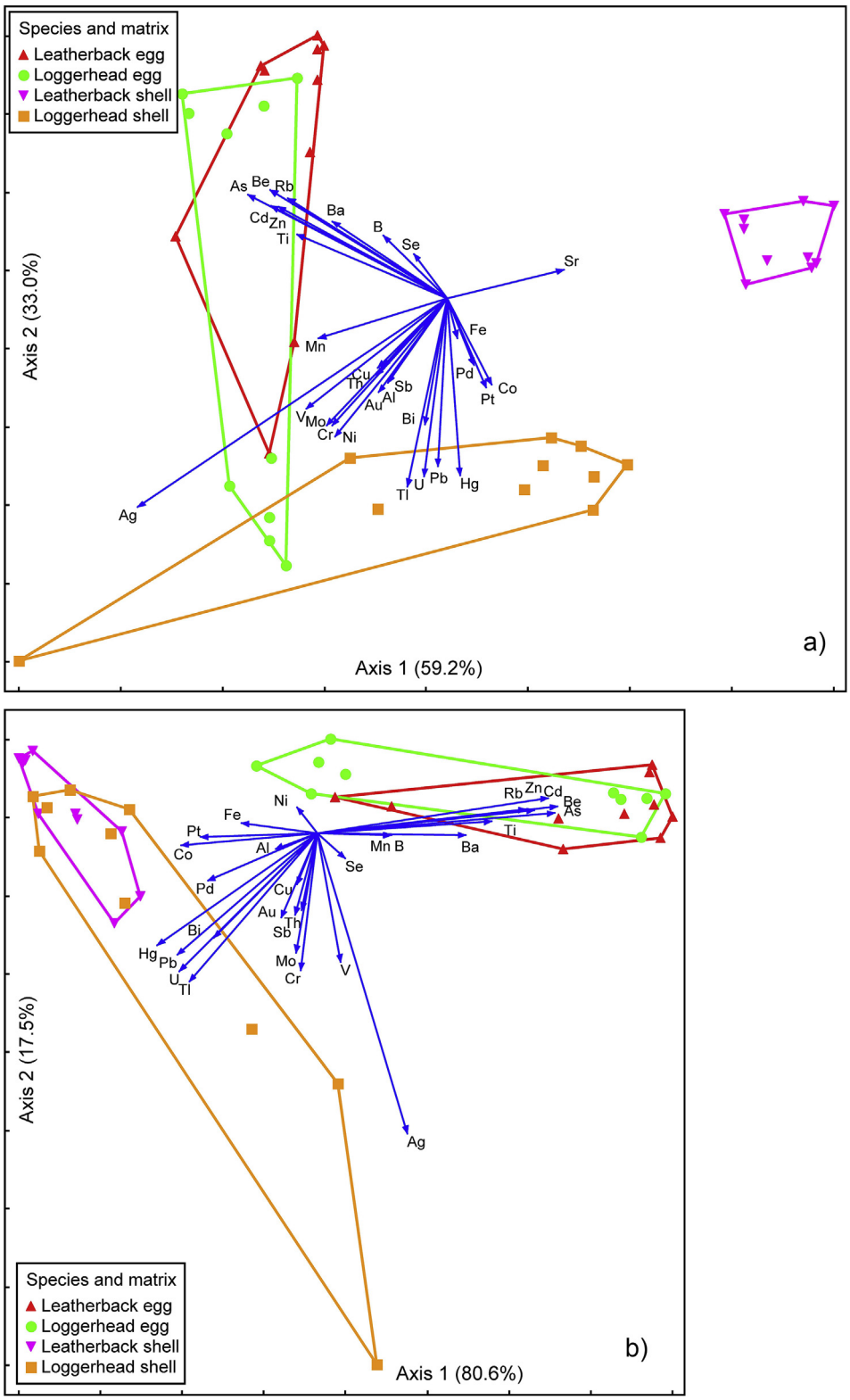


Fig. 3. a) Non-metric multidimensional scaling of relativized metal and metalloid concentrations in loggerhead and leatherback eggshells and egg contents. b) Non-metric multidimensional scaling of relativized metal and metalloid concentrations in loggerhead and leatherback eggshells and egg contents without Sr.

mean Sr concentrations were about the same in egg contents of both species, but more than double in the leatherback eggshells (Table 1). Strontium is a Ca mimic (both are divalent alkaline earth metals). Due to its similarity to calcium, Sr accumulates in milk and bone (Nordberg et al., 2015).

Leatherback sea turtle eggshells contain 90% of the total Ca of the egg (Bilinski et al., 2001). Sr seems to accumulate in the eggshell of leatherback sea turtles due to its high Ca content. The most likely

explanation is that leatherback sea turtles incorporate strontium within the eggshell along with calcium due to ionic similarity and availability. We found one study that specifically commented on Sr and turtles. Although the Sr concentrations in juvenile green turtles from Hong Kong were the same as juveniles from Japan, adult green turtle muscle and liver from Hong Kong had 30 and 100 times higher concentrations, respectively, than from Japan (Lam et al., 2004). Local geography, pollution, and age were given as possible explanations but nothing conclusive. Strontium can accumulate in some scyphozoan jellyfishes species (Templeman and Kingsford, 2012), and this may partially explain our observations, since leatherback turtles feed almost exclusively on cnidarians (Eckert et al., 2012).

Calcium occurs in seawater at 400 mg/kg, and Sr at 8 mg/kg (Garison, 2013). Why the leatherback eggshells would have so much more Sr in the eggshells relative to the contents is unknown, given that loggerhead turtle eggshells had significantly higher concentrations of seventeen other elements. The Ca/Sr ratios also need to be determined. The influence of Sr on elemental compositional patterns will be discussed in section 4.5.

4.5. Toxic effects

The developing embryo is likely one of the most sensitive life stages susceptible to xenobiotic disturbance (Hopkins et al., 1999; Brasfield et al., 2004). Low leatherback hatching success in Florida was correlated with high Hg and low Se concentrations (Perrault et al., 2011). Selenium aids in the removal of Hg. Mercury is probably detoxified by the liver of the animal through insoluble mercury–selenium compounds that are then excreted (Perrault et al., 2011). The relatively high Se in both egg contents and eggshells from South African marine turtles may thus explain the relatively low Hg concentrations in the egg contents, or low levels in prey.

As explained above, it is often not possible to determine the clutch sequence from which the eggs were taken. Doubling the mean mercury concentrations for the egg contents to accommodate variations between clutches results in concentrations of 0.0010 and 0.0012 mg/kg wm for the leatherback and loggerhead eggs from South Africa, respectively. This is well below the mean of 0.048 mg/kg in egg yolk sacks of leatherback eggs from Florida (Perrault et al., 2011) with low hatching success. However, it is possible that yolk sacs remaining after hatching have concentrated levels of metallic elements, as was the case for POPs (Alava et al., 2006). Interestingly though, was that liver Se and liver Se/Hg ratios corresponded positively with hatching success in the Florida study. Even doubling the maximum concentrations for our leatherback and loggerhead egg content to concentrations of 0.0017, and 0.0016 mg/kg wm, respectively is still an order of magnitude less than the mean from Florida.

Endocrine disruption caused by metals and organic pollutants is a possibility. Endocrine disruptors may influence sex ratios during development without mortality, added to possible complication from climate change when dealing with temperature-dependant sex determination in reptiles (Oberdörster and Cheek, 2000; Cheek, 2006; Ikonomopoulou et al., 2009; Howard et al., 2014; Finlayson et al., 2016; Jensen et al., 2018). Such complex interactions between climate change and toxicity needs careful investigation.

Although very little experimental information is available for toxic effects of metals and metalloids on leatherback turtles (Finlayson et al., 2016), turtles are considered to be at risk of biomagnification of potentially toxic contaminants (Lam et al., 2006a,b; Guirlet et al., 2008; Roe et al., 2011). From literature, we can compare with the following:

- The mean and maxima of Hg for both marine turtle species are well below its toxic reference values (TRV) in bird egg contents (Meyer et al., 2014): the mercury TRV is 2 mg/kg dm, while the highest concentration in the eggs of the present study was 0.0012 mg/kg dm for both loggerhead and leatherback egg contents. As we suggested above, and to accommodate mercury's variation between clutches, even double the Hg concentrations remained well below the bird egg TRV for Hg.
- The bird egg TRV for Se is 8 mg/kg dm; the highest concentration we measured was 7.7 mg/kg dm in a loggerhead egg.
- Based on hazard quotients (HQ) calculated by Lam et al. (2006a,b), the worst- and best-case scenario HQs for Se are 0.2 and 24.5 mg/kg wm, respectively. For loggerhead and leatherback eggs, all eggs exceeded the worst-case scenario, but not the best-case scenario (range 1.7–4.6 mg/kg wm for loggerhead eggs, and 1.3–4.6 mg/kg wm for leatherback eggs).
- The TRV for copper at 10–20 mg/kg dm was reached and exceeded in two loggerhead eggs at 16 mg/kg dm and 30 mg/kg dm.
- The bird egg TRV for Sr of 66–73 mg/kg dm was exceeded by all but one leatherback egg, and six of the ten loggerhead eggs for the lowest level in the range.
- Based on hazard quotients (HQ) calculated by Lam et al. (2006a,b), the worst- and best-case scenario HQs for Pb are <0.1 and 0.2 mg/kg wm, respectively. For loggerhead and leatherback, none of the eggs exceeded any of the two scenarios (range 0.00053–0.0011 mg/kg wm for loggerhead eggs, and 0.000006–0.031 mg/kg wm for leatherback eggs).
- Based on hazard quotients (HQ) calculated by Lam et al. (2006a,b), the worst- and best-case scenario HQs for Ni are 26.4 and 4.0 mg/kg wm, respectively. For loggerhead and leatherback, none of the eggs exceeded any of the two scenarios (range 0.14–6.7 mg/kg wm for loggerhead eggs, and 0.07–1.6 mg/kg wm for leatherback eggs).

Copper and selenium may thus pose a risk to the turtles breeding on South African shores. Based on the high hatching success of these sea turtles (Hughes, 1974), it seems that the concentrations of metals and metalloids (including mercury) may not be affecting hatching negatively. However, the latest emergence success data are decades old; more recent and long-term monitoring data would be needed for confirmation. At least Cu, Hg, Sr, Se, and Ni should be monitored but current technology allows more elements to be analysed. Thallium is more toxic than the aforementioned elements, is attracting more attention (John Peter and Viraraghavan, 2005 and citations), and should be included in this list.

4.6. Can eggshells be used as a proxy for egg contents?

Eggs are excellent biological indicators of pollutants in birds (Evers et al., 2003; Bouwman et al., 2015a,b) and reptiles (Bouwman et al., 2014; du Preez et al., 2018; and references in Table 1) as they are relatively easily sampled, represents an entire organism, have a consistent composition, and collection from large clutches presumably have negligible effects on populations. There is, however, also the option to sample eggshells of hatched eggs rather than viable eggs and thereby reduce the impact on threatened species. Feather concentrations are often used as a proxy for body burden in birds (Dauwe et al., 2000; Anasara-Ross et al., 2013), but feathers are conspicuously absent from turtles. In this section, we will investigate the viability of using turtle eggshells as a proxy for egg contents.

A study from Hong Kong regressed concentrations in green turtle eggs with eggshells and found significant associations for Ag, Se, Zn, Hg, and Pb (Lam et al., 2006a,b). Magnesium in egg contents and eggshells were positively associated in red-eared slider turtle (terrapin) (*Trachemys scripta*) from the USA (Burger and Gibbons, 1998). In the present study, egg contents and eggshell concentrations were positively correlated only for Se, Sb, and Ba in leatherback eggs, and Zn in loggerhead eggs. Thallium egg and eggshell concentrations were negatively associated in loggerheads. However, due to the large number of correlations (60), the likelihood of significant correlations by chance is rather high. Combining our findings with others (Burger and Gibbons, 1998; Lam et al., 2006a,b), we discount haphazard relationships between egg contents and eggshells; only very good relationships established over a long period and with many more paired egg contents-eggshell measurements will support using eggshells only to infer contaminant levels in egg contents.

Direct comparisons between and within species of egg contents and eggshell concentrations showed little correspondence (section 4.1). The %CV values were quite large (Table 2), and larger in eggshells than in egg contents. The differences between the means for eggshell and egg contents were significant for leatherback eggs (Wilcoxon matched-pairs, signed rank tests, $p < 0.0001$), and marginal (Wilcoxon matched-pairs, signed rank tests, $p = 0.0724$) for loggerheads. Interestingly, Ag showed the greatest variation in both species for both eggshell and egg contents Table 2). Silver is therefore a good example of a toxic element that is not likely to be useful for extrapolation between eggshell and egg contents. Cross-species comparisons of respective matrixes showed significant differences in %CVs between egg contents (Mann-Whitney, $p = 0.0078$), but not for the eggshells (Table 2).

It can be argued however, that a 'fingerprint' or pattern analyses (using relative proportions) will be more appropriate. Firstly, we used MRPP to compare elemental profiles between eggshells and egg contents within each species. The large negative *T* values and small *A* values (Table 2, under the MRPP column heading) indicates little correspondence between profiles, with very small likelihood that these differences were by chance. Again, overall, elemental patterns did not agree between eggshell and egg contents within each species. As will be made clear why in section 4.3, we removed Sr. Still, there was no agreement in patterns (Table 2, column heading MRPP no Sr).

Secondly, we used multivariate visualization technique (NMS) of relativized data to look at possible pattern correspondence between eggshell and egg content. For both species (Fig. 2a and b), the convex hulls that delineate the outer sample ordination points did not overlap. For leatherbacks (Fig. 2a) the difference was quite marked, with higher proportions of elements such as As, Cd, Be, and Zn characterising egg contents (vectors pointing towards the right), and higher proportions of Hg, U, Ti, and Pb characteristic of the shell. The small convex hull for leatherback eggshells (to the left) shows good congruence for those elements that occur in higher proportions here. For leatherbacks, there is only slight overlap (Fig. 2b).

As for leatherbacks (Fig. 2a), the loggerhead eggshells were also characterised by higher proportions of Hg, Pb, and U, but not for Ti (Fig. 2b). Arsenic, Be, Rb, Zn, and Cd also characterised the loggerhead shells, as they did for leatherbacks. The long Ag vector in Fig. 2b associates with a single eggshell at the top of the ordination. This egg therefore had a very high Ag concentration relative to all other elements in the egg contents, and likely explains the strong explanatory power of the second axis (42.3%, compared with 10.7% for the leatherbacks).

When comparing Sr and Hg in both species, Hg and Sr ordinated much in the same direction towards the leatherback eggshell – therefore, a higher proportion of Hg is accompanied by a higher proportion of Sr. In the loggerhead, Hg also associates with the shell, but Sr is almost perpendicular towards a sub-cluster of eggshells (to be discussed below).

The two matrixes we analysed—eggshells and egg contents—have two complementary but different functions. The shells serves as containment, protection, source of nutrients, and as a mechanism to regulate gas exchange for the egg contents, while the egg contents (ova, lipid-rich yolk, and protein-rich albumin) supports the development of the embryo and foetus (Deeming and Ferguson, 1991). That there would be differences in proportional composition of the elements between the two matrixes is therefore to be expected.

Based on direct comparisons of concentrations, the analysis of the %CVs, the separation between pattern envelopes for both species, and the large differences in vector lengths of the elements, there is therefore little scope for using eggshells as proxy for egg contents for both species.

4.7. Comparisons between species

But what about cross-species comparisons? Can the eggshells of the more abundant loggerheads be used to predict the concentrations in endangered leatherbacks? This is shown in NMS ordinations as Fig. 3a and b. With all elements included, there was no congruence between the elemental patterns of eggshells of both species, but quite some congruence between the egg contents of both species (NMS ordinations only with eggshell-eggshell data, or only egg content-egg content data, showed very much the same congruence and differences as in Fig. 3b – not shown here). Very prominently, Sr showed a very strong association with loggerhead eggshells and an anomalous orientation in respect to all other elemental vectors. Based on the additional discussion above (Section 4.1) about the significantly higher Sr concentrations in leatherback shells, we deleted the Sr data from the dataset and ordinated again (Fig. 3b). This changed the picture dramatically, with both eggshell pattern envelopes now congruent. The same As, Be, Cd, Zn, Rb, and Ti co-variation with egg contents is now demonstrated for both species, as well as Tl, U, Pb, and Hg for the shells, apparent from Fig. 2a and b.

Fig. 3a and b shows three things; the elemental patterns in eggs from both species overlap, the elemental patterns in the eggshells of both species overlap, but the patterns for eggs and eggshells do not. Close examination also shows possible sub-groupings. Especially for egg contents, there seems to be two groups at opposite ends of the overlapping convex hulls, and to a lesser extent, this is also apparent for the shells. This may be due to individuals being either coastal or oceanic, evidence of which have been presented by satellite telemetry for both species (Robinson et al., 2016). If this were the case, the picture becomes more complicated as it seems difficult to distinguish maternal range preferences from breeding females on the beach without satellite tracking data, or nests alone.

Deme discrimination might be able to distinguish between oceanic and coastal individuals, even based on eggshell elemental composition alone. Based on metal patterns, indications of demes for loggerheads breeding in North Carolina, USA, (Stoneburner et al., 1980), and green turtles from Baja, California Peninsula (Mexico) was found (Talavera-Saenz et al., 2007). Alava et al. (2011) found similar indications based on POPs for loggerheads, also from the east coast of the USA.

More data are needed on elemental levels in marine turtles, supplemented with persistent organic pollutant analyses that normally have much slower turnover in bodies than metals and metalloids (Fisk et al., 2001) to discriminate between demes.

5. Synthesis and conclusion

In general, South African turtles had lower concentrations of metals and metalloids in their eggs and eggshells than reported by other studies. Loggerheads generally are coastal and this might explain their higher concentrations of pollutants due to river runoff, marine debris, ship, and harbour pollution affecting prey species, compared with the more oceanic leatherback that feeds at a lower trophic level, although allosteric size factors may also play a role.

Compared with Sr in egg contents, both species had concentrations in their eggshells an order of magnitude higher. Furthermore, the mean Sr concentrations (a Ca mimic) in the leatherback eggshells were more than double the mean in loggerhead shells. We could not quite explain this discrepancy, and suggest that further work is needed in this regard. Strontium is incorporated in some plastics as a flame retardant (Golloch and Siegmund, 1997), and its ingestion by leatherbacks mistaking plastics for jellyfish might be a source (Mrosovsky et al., 2009), in addition to Sr occurring in jellyfish statotoliths (Mooney and Kingsford, 2012).

Mercury concentrations in egg contents were low compared with available data (Table 1), and also did not exceed mercury's TRV for bird eggs. The TRVs for Sr and Cu were exceeded. However, the hatching success of the South African leatherbacks (based on old data) is such to suggest that there is very little toxicological influence, although sub-lethal effects and mixture effects (Finlayson et al., 2016) should not be ignored. In addition to organic compounds such DDE, some metals are also known endocrine disruptors and may influence sex ratios during development without affecting hatching success. There is also the added possible complication from climate change (Oberdörster and Cheek, 2000; Cheek, 2006; Finlayson et al., 2016; du Preez et al., 2018; Jensen et al., 2018).

Due to their toxic threat, we suggest that at least Hg, Al, Pb, As, Co, Cd, Cu, V, Ni, Zn, Mn, and Sr, be monitored. We also suggest adding Au, Ba and Tl to this list, since it seems to be receiving more attention from terrestrial and marine perspectives (Van der Schyff et al., 2016; Wise et al., 2011). Based on our observations of Sr, it seems prudent to add the remaining alkaline earth metals, Ca, Ra, Be, Mg. Data on organic contaminants will also be very useful, as newer types of persistent organic pollutants such as brominated flame retardants and perfluorinated compounds have been detected in African penguin *Spheniscus demersus* eggs from the South African coast (Bouwman et al., 2015b). Economic development and other forces such as conflict and population growth around the Indian Ocean basin (Kaplan, 2010) are likely to increase pollutant releases (Bouwman et al., 2015a) and trends need to be monitored.

As to the question whether eggshells can be used as a proxy for each species and between species, we suggest that the differences in concentrations between species and the results of the MRPP and NMS analyses are not convincing enough to use only eggshells. Although there were overlap in eggshell and egg content patterns between the species, there was no overlap between eggshells and egg contents. Predicting concentrations at the site of toxicological action, the egg contents, will not be viable unless very wide uncertainty factors are employed. Eggshells may perhaps be used as a warning for long-term concentration trends in the oceanic background.

Maternal turtles serve as 'active samplers' that often return to the same general location to breed (allowing biological material collection)—something that is not practical with marine mammals or elasmobranchs. In addition, elemental patterns may also indicate the feeding ranges of the maternal animals. However, egg content data of a limited number collected regularly would possibly serve the same purpose with much more confidence with little impact. Continued regular sampling and analyses of eggshells and egg contents (and possibly blood) of both species would provide a convincing and easily obtainable trend and warning of pollutant changes in the oceanic ranges frequented by the South African turtles.

Acknowledgements

We thank Chris Nolte, Linda Harris, Anton Cloete and Diane Le Gouvello for their help with the collection of the turtle eggs. We thank Bianca Michelle Vogt, Daniël van Aswegen and Veronica van der Schyff for their help in the laboratory. We also thank three anonymous reviewers. We thank the National Research Foundation (NRF) of South Africa for funding. Opinions expressed and conclusions arrived at are those of the authors and are not necessarily to be attributed to the NRF.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.chemosphere.2018.01.106.

References

Aguirre, A.A., Balazs, G.H., Zimmerman, B., Galey, F.D., 1994. Organic contaminants and trace metals in the tissues of green turtles (*Chelonia mydas*) afflicted with fibropapillomas in the Hawaiian islands. Mar. Pollut. Bull. 28, 109–114.

Anasara-Ross, T.M., Ross, M.J., Wepener, V., 2013. The use of feathers in monitoring bioaccumulation of metals and metalloids in the South African endangered African grass-owl (*Tyto capensis*). Ecotoxicology 22, 1072–1083.

Alava, J., Keller, J., Kucklick, J., Wyneken, J., Crowder, L., Scott, G., 2006. Loggerhead sea turtle (*Caretta caretta*) egg yolk concentrations of persistent organic pollutants and lipid increase during the last stage of embryonic development. Sci. Total Environ. 367, 170–181.

Alava, J.J., Keller, J.M., Wyneken, J., Crowder, L., Scott, G., Kucklick, J.R., 2011. Geographical variation of persistent organic pollutants in eggs of threatened loggerhead sea turtles (*Caretta caretta*) from southeastern United States. Env. Toxicol. Chem. 30, 1677–1688.

Bels, V.L., Davenport, J., Renous, S., 1998. Food ingestion in the estuarine turtle *Malaclemys terrapin*: Comparison with the marine leatherback turtle *Dermochelys coriacea*. J. Mar. Biol. Assoc. U. K. 78, 953–972.

Bicho, R.C., Mendonca, V.M., Al Kiyumi, A.A., Al Saady, S.M., Al Habsi, A., Al Kindi, A., Mahmoud, I.Y., Kalb, H., Rohde, A.S., Gayheart, K., Shanker, K., 2008. Accumulation in livers and excretion through eggs of heavy metals in a nesting population of green turtles, Chelonia mydas, in the Nw Indian Ocean. NOAA Tech 582, 59.

Bilinski, J.J., Reina, R.D., Spotila, J.R., Paladino, F.V., 2001. The effects of nest environment on calcium mobilization by leatherback turtle embryos *Dermochelys coriacea* during development. Comp. Biochem. Physiol. 130, 151–162.

Bouwman, H., Booyens, P., Govender, D., Pienaar, D., Polder, A., 2014. Chlorinated, brominated, and fluorinated organic pollutants in nile crocodile eggs from the kruger national park, South Africa. Ecotoxicol. Environ. Saf. 104, 393–402.

Bouwman, H., Govender, D., Underhill, L., Polder, A., 2015b. Chlorinated, brominated and fluorinated organic pollutants in African Penguin eggs: 30 years since the previous assessment. Chemosphere 126, 1–10.

Bouwman, H., Kylin, H., Choong Kwet Yive, N.S., Tatayah, V., Løken, K., Skaare, J.U., Polder, A., 2015a. First report of chlorinated and brominated hydrocarbon pollutants in marine bird eggs from an oceanic Indian Ocean island. Environ. Res. 118, 53–64.

Brasfield, S.M., Bradham, K., Wells, J.B., Talent, L.G., Lanno, R.P., Janz, D.M., 2004. Development of a terrestrial vertebrate model for assessing bioavailability of cadmium in the fence lizard (*Sceloporus undulatus*) and in ovo effects on hatchling size and thyroid function. Chemosphere 54, 1643–1651.

Burger, J., 2002. Food chain differences affect heavy metals in bird eggs in Barnegat Bay, New Jersy. Env. Res. Sect A 90, 33–39.

Burger, J., Gibbons, J.W., 1998. Trace elements in egg contents and egg shells of slider turtles (*Trachemys scripta*) from the Savannah River site. Arch. Environ. Contam. Toxicol. 34, 382–386.

Çelik, A., Kaska, Y., Bağ, H., Aureggi, M., Semiz, G., Kartal, A.A., Elçi, L., 2006. Heavy metal monitoring around the nesting environment of green sea turtles in Turkey. Water Air Soil Pollut. 169, 67–79.

Cheek, A.N., 2006. Subtle sabotage: endocrine disruption in wild populations. Biol. Ther. 54, 1–19.

Cortes-Gomez, A.A., Fuentes-Mascorro, G., Romero, D., 2014. Metals and metalloids in whole blood and tissues of olive ridley turtles (*Lepidochelys olivacea*) from La escobilla beach (oaxaca, Mexico). Mar. Pollut. Bull. 89, 367–375.

D'Ilioe, S., Mattei, A., Blasi, M.F., Alimonti, A., Bogialli, S.C., 2011. The occurrence of chemical elements and POPs in loggerhead turtles (*Caretta caretta*): an overview. Mar. Pollut. Bull. 62, 1606–1615.
Dauwe, T., Bervoets, L., Blust, R., Pinxten, R., Eens, M., 2000. Can excrement and feathers of nestling songbirds be used as biomonitors for heavy metal pollution? Arch. Environ. Contam. Toxicol. 39, 541–546.
Deeming, D.C., Ferguson, M.W.J., 1991. Egg Incubation: its Effects on Embryonic Development in Birds and Reptiles. Cambridge University Press, Cambridge.
du Preez, M., Govender, D., Kylin, H., Bouwman, H., 2018. Metallic elements in Nile crocodile eggs from the Kruger national Park, South Africa. Ecotoxicol. Environ. Saf. 148, 930–941.
Eckert, K.L., Wallace, B.P., Frazier, J.G., Eckert, S.A., Pritchard, P.C.H., 2012. Synopsis of the Biological Data on the Leatherback Sea Turtle (Dermochelys Coriacea). US Fish and Wildlife Service. Biological Technical Publication BTP-R4015–2012.
Ehsanpour, M., Afkhami, M., Khoshnood, R., Reich, K.J., 2014. Determination and maternal transfer of heavy metals (Cd, Cu, Zn, Pb and Hg) in the hawksbill sea turtle (*Eretmochelys imbricata*) from a nesting colony of Qeshm Island, Iran. Bull. Environ. Contam. Toxicol. 92, 667–673.
Evers, D.C., Taylor, K.M., Major, A., Taylor, R.J., Poppenga, R.H., Scheuhammer, A.M., 2003. Common loon eggs as indicators of methylmercury availability in North America. Ecotoxicology 12, 69–81.
Finlayson, K.A., Leusch, F.D.L., van der Merwe, J.P., 2016. The current state and future research directions of marine turtle toxicology research. Environ. Int. 94, 113–123.
Fisk, A.T., Hobson, K.A., Norstrom, N.J., 2001. Influence of chemical and biological factors on trophic transfer of persistent organic pollutants in the North Water Polynya marine food web. Environ. Sci. Technol. 35, 732–738.
Garison, T., 2013. Oceanography: an Invitation to Marine Science. National Geographic Learning. Brooks Cole Cengage Learning.
Gardner, S.C., Fitzgerald, S.L., Vargas, B.A., Rodríguez, L.M., 2006. Heavy metal accumulation in four species of sea turtles from the Baja California peninsula, Mexico. Biometals 19, 91–99.
Greenfield, R., Wepener, V., Degger, N., Brink, K., 2011. Richards Bay Harbour: metal exposure monitoring over the last 34 years. Mar. Pollut. Bull. 62, 1926–1931.
Godly, B.J., Thompson, D.R., Furness, R.W., 1999. Do heavy metal concentrations pose a threat to marine turtles from the Mediterranean Sea? Mar. Pollut. Bull. 38, 497–502.
Golloch, A., Siegmund, D., 1997. Sliding spark spectroscopy – rapid survey analysis of flame retardants and other additives in polymers. J. Anal. Chem. 358, 804–811.
Guirlet, E., Das, K., Girondot, M., 2008. Maternal transfer of trace elements in leatherback turtles (*Dermochelys coriacea*) of French Guiana. Aquat. Toxicol 88, 267–276.
Hopkins, W.A., Rowe, C.L., Congdon, J.D., 1999. Elevated trace element concentrations and standard metabolic rate in banded water snakes (*Nerodia fasciata*) exposed to coal combustion wastes. Env. Tox. Chem. 18, 1259–1263.
Howard, R., Bell, I., Pike, D.A., 2014. Thermal tolerances of sea turtle embryos: current understanding and future directions. Endanger. Species Res. 26, 75–86.
Hughes, G.R., 1974. The Sea Turtles of South-east Africa. Vol. II. The Biology of the Tongaland Loggerhead Turtle Caretta caretta L. With Comments on the Leatherback Turtle Dermochelys Coriacea L. and the Green Turtle Chelonia mydas L. in the Study Region. Investigational Report No. 36. Oceanographic Research Institute, Durban, South Africa, p. 96.
Hughes, G.R., 1996. Nesting of the leatherback turtle (*Dermochelys coriacea*) in Tongaland, KwaZulu-Natal, South Africa, 1963-1995. Chelonian Conserv. Biol. 2, 153–158.
Ikonomopoulou, M.P., Olzowy, H., Hodge, M., Bradley, A.J., 2009. The effect of organochlorines and heavy metals on sex steroid-binding proteins in vitro in the plasma of nesting green turtles, *Chelonia mydas*. J. Comput. Chem. B 179, 653–662.
Ikonomopoulou, M.P., Olszowy, H., Limpus, C., Francis, R., Whittier, J., 2011. Trace element concentrations in nesting flatback turtles (*Natator depressus*) from Curtis Island, Queensland, Australia. Mar. Environ. Res. 71, 10–16.
IUCN, 2013. Dermochelys Coriacea (Southwest Indian Ocean Subpopulation). http://www.iucnredlist.org/details/46967863/0 (Accessed 14 August 2016).
IUCN, 2015. Caretta caretta (South West Indian Ocean Subpopulation). http://www.iucnredlist.org/details/84199475/0 (Accessed 14 August 2016).
Jakimska, A., Konieczka, P., Skora, K., Namiesnik, J., 2011. Bioaccumulation of metals in tissues of marine animals, Part I: the role and impact of heavy metals on organisms. Pol. J. Environ 20, 1117–1125.
Järup, L., 2013. Hazards of heavy metal contamination. Br. Med. Bull. 68, 167–182.
John Peter, A.L., Viraraghavan, T., 2005. Thallium: a review of public health and environmental concerns. Environ. Int. 31, 493–501.
Jensen, M.P., Allen, C.D., Eguchi, T., Bell, I.P., LaCasella, E.L., Hilton, W.A., Hof, C.A.M., Dutton, P.H., 2018. Environmental warming and feminization of one of the largest sea turtle populations in the world. Curr. Biol. 28, 154–159.
Kaplan, R.D., 2010. Monsoon: the Indian Ocean and the Future of American Power. Random House, New York.
Kaska, Y., Furness, R.W., 2001. Heavy metals in marine turtle eggs and hatchlings in the Mediterranean. Zool. Middle East 24, 127–132.
Lam, J.C.W., Tanabe, S., Chan, S.K.F., Yuen, E.K.W., Lam, M.H.W., Lam, P.K.S., 2004. Trace element residues in tissues of green turtles (*Chelonia mydas*) from South China waters. Mar. Pollut. Bull. 48, 164–192.
Lam, J.C.W., Tanabe, S., Chan, S.K.F., Lam, M.H.W., Martin, M., Lam, P.K.S., 2006a. Levels of trace elements in green turtle eggs collected from Hong Kong: evidence of risks due to selenium and nickel. Environ. Pollut. 144, 790–801.
Lam, J.C., Tanabe, S., Chan, S.K., Lam, M.H., Martin, M., Lam, P.K., 2006b. Levels of trace elements in green turtle eggs collected from Hong Kong: evidence of risks due to selenium and nickel. Environ. Pollut. 144, 790–801.
Luschi, P., Casale, P., 2003. Movement patterns of marine turtles in the Mediterranean Sea: a review. Ital. J. Zool. 81, 478–495.
Luschi, P., Lutjeharms, J.R.E., Lambardi, P., Mencacci, R., Hughes, G.R., Hays, G.C., 2006. A review of migratory behaviour of sea turtles off southeastern Africa. S. Afr. J. Sci. 102, 51–58.
Mooney, C.J., Kingsford, M.J., 2012. Sources and movements of *Chironex fleckeri* medusae using statolith elemental chemistry. Hydrobiologia 690, 69–277.
Marco, A., Lopez-Vicente, M., Perez-Mellado, V., 2004. Arsenic uptake by reptile flexible-shelled eggs from contaminated nest substrates and toxic effect on embryos. Bull. Environ. Contam. Toxicol. 72983–72990.
McCune, B., Grace, J.B., 2002. Analysis of Ecological Communities. MjM Software Design, Gleneden Beach, Oregon.
Meyer, C.B., Schlekat, T.H., Walls, S.J., Iannuzzi, J., Souza, M.J., 2014. Evaluating risks to wildlife from coal fly ash incorporating recent advances in metals and metalloids risk assessment. Integr. Environ. Assess. Manag 11, 67–79.
Mrosovsky, M., Ryan, G.D., James, M.C., 2009. Leatherback turtles: the menace of plastic. Mar. Pollut. Bull. 58, 287–289.
Nagle, R.D., Rowe, C.L., Congdon, J.D., 2001. Accumulation and selective maternal transfer of contaminants in the turtle *Trachemys scripta* associated with coal ash deposition. Arch. Environ. Contam. Toxicol. 40, 531–536.
Nel, R., Punt, A.E., Hughes, G.R., 2013. Are coastal protected areas always effective in achieving population recovery for nesting sea turtles? PLoS One 8 (5), e63525. https://doi.org/10.1371/journal.pone.0063525.
Ng, J.C., Juhasz, A., Smith, E., Naidu, R., 2013. Assessing the bioavailability and bioaccessibility of metals and metalloids. Environ. Sci. Poll. Res. 22, 8802–8825.
Newman, M.C., Heagler, M.G., 1991. Allometry of metal bio accumulation. In: Newman, M.C., McIntosh, A.W., Lewis Publishers, Inc (Eds.), Metal Ecotoxicology, Concepts and Applications. Michigan, Chelsea, p. 399.
Nicolau, L., Marçalo, A., Ferreira, M., Sá, S., Vingada, J., Eira, C., 2015. Ingestion of marine litter by loggerhead sea turtles, *Caretta caretta*, in Portuguese continental waters. Mar. Pollut. Bull. 12, 25–32.
Nordberg, G.F., Fowler, B.A., Nordberg, M., 2015. Handbook on the Toxicology of Metals, fourth ed. Elsevier press, London, pp. 1–1383.
Oberdörster, E., Cheek, A.O., 2000. Gender benders at the beach: endocrine disruption in marine and estuarine organisms. Environ. Toxicol. Chem. 20, 23–36.
Páez-Osuna, F., Calderón-Campuzano, M.F., Soto-Jiménez, M.F., Ruelas-Inzunza, J.R., 2010. Trace Metals (Cd, Cu, Ni, and Zn) in blood and eggs of the sea turtle *Lepidochelys olivacea* from a nesting colony of Oaxaca, Mexico. Arch. Environ. Contam. Toxicol. 59, 632–641.
Papi, F., Luschi, P., Crosio, E., Hughes, G.R., 1997. Satellite tracking experiments on the navigational ability and migratory behaviour of the Loggerhead Turtle *Caretta caretta*. Mar. Biol. 129, 215–220.
Perrault, J., Wyneken, J., Thompson, L.J., Johnson, C., Miller, D.L., 2011. Why are hatching and emergence success low? Mercury and selenium concentrations in nesting leatherback sea turtles (*Dermochelys coriacea*) and their young in Florida. Mar. Pollut. Bull. 62, 1671–1682.
Perrault, J.R., Wyneken, J., Page-Karjian, A., Merrill, A., Miller, D.L., 2014. Seasonal trends in nesting leatherback turtle (*Dermochelys coriacea*) serum proteins further verify capital breeding hypothesis. Conserv. Physiol. 2, 1–15.
Robinson, N.J., Morreale, S.J., Nel, R., Paladino, F.V., 2016. Coastal leatherback turtles reveal conservation hotspot. Sci. Rep. 6, 37851. https://doi.org/10.1038/srep37851.
Roe, J.H., Sill, N.S., Columbia, M.R., Paladino, F.V., 2011. Trace metals in eggs and hatchlings of Pacific leatherback turtles (*Dermochelys coriacea*) nesting at Playa Grande, Costa Rica. Chelonian Conserv. Biol. 10, 3–9.
Ross, D.A., Guzmán, H.M., Van Hinsberg, V.J., Potvin, C., 2016. Metal contents of marine turtle eggs (*Chelonia mydas; Lepidochelys olivacea*) from the tropical eastern pacific and the implications for human health. J. Environ. Sci. Health, Part B 51 (10), 675–687. https://doi.org/10.1080/03601234.2016.1191888.
Sahoo, G., Mohapatra, B.K., Sahoo, R.K., Mohanti-Hejmadi, P., 1996. Contrasting ultrastructures in the eggshells of olive ridley turtles, Lepidochelys olivacea, from Gahirmatha. Orissa. Curr. Sci 246–249.
Sakai, H., Ichihashi, H., Suganuma, H., Tatsukawa, R., 1995. Heavy metal monitoring in sea turtles using eggs. Mar. Pollut. Bull. 30, 347–353.
Sakai, H., Saeki, K., Ichihashi, H., Suganuma, H., Tanabe, S., Tatsukawa, R., 2000. Species-specific distribution of heavy metals in tissues and organs of loggerhead turtle (*Caretta caretta*) and green turtle (*Chelonia mydas*) from Japanese coastal waters. Mar. Pollut. Bull. 40, 701–709.
Sola, S., Prat, N., 2006. Monitoring metal and metalloid bioaccumulation in Hydropsyche (Trichoptera, Hydropsychidae) to evaluate metal pollution in a mining river. Whole body versus tissue content. Sci. Total Environ. 359, 221–231.
Storelli, M.M., Marcotrigiano, G.O., 2003. Heavy metal residues in tissues of marine turtles. Mar. Pollut. Bull. 46, 397–400.
Stoneburner, D.L., Nicora, M.N., Blood, E.R., 1980. Heavy metals in loggerhead sea turtle eggs (*Caretta caretta*): evidence to support the hypothesis that demes exist in the western Atlantic population. J. Herpetol. 4, 171–175.
Talavera-Saenz, A., Gradner, S.C., Rodrigiez, R.R., Vargaa, B.A., 2007. Metal profiles used as environmental markers of green turtle (*Chelonia mydas*) foraging resources. Sci. Total Environ. 373, 94–102.

Templeman, M.A., Kingsford, M.J., 2012. Variation in soft tissue chemistry among scyphozoan and cubozoan jellyfishes from the Great Barrier Reef, Australia. Hydrobiologia 690, 279–290.

Tucek, J., Nel, R., Girondot, M., Hughes, G., 2014. Age-size relationship at reproduction of South African female loggerhead turtles Caretta caretta. Endanger. Species Res. 23, 167–175.

van de Merwe, J.P., Hodge, M., Olszowy, H.A., Whittier, J.M., Ibrahim, K., Lee, S.Y., 2009. Chemical contamination of green turtle (*Chelonia mydas*) eggs in peninsular Malaysia: implications for conservation and public health. Environ. Health Perspect. 117, 1397–1401.

van de Merwe, J.P., Hodge, M., Whittier, J.M., Ibrahim, K., Lee, S.Y., 2010. Persistent organic pollutants in the green sea turtle Chelonia mydas: nesting population variation, maternal transfer, and effects on development. Mar. Ecol. Prog. Ser 403, 269–278.

van der Schyff, V., Pieters, R., Bouwman, H., 2016. The heron that laid the golden egg: metals and metalloids in ibis, darter, cormorant, heron, and egret eggs from the Vaal River catchment, South Africa. Environ. Monit. Assess. 188, 372–379.

Villa, C.A., Flint, M., Bell, I., Limpus, C.J., Gaus, C., 2017. Trace element reference intervals in the blood of healthy green sea turtles to evaluate exposure of coastal populations. Env. Pollut 220, 1465–1476.

Wepener, V., Degger, N., 2012. Status of marine pollution research in South Africa (1960-present). Mar. Pollut. Bull. 64, 1508–1512.

Wise, J.P., Thompson, W.D., Wise, S.S., LaCerte, C., Wise, J., Gianos, C., Perkins, C., Zheng, T., Benedict, L., Mason, M.D., Payne, R., Kerr, I., 2011. A global assessment of gold, titanium, strontium and barium pollution using sperm whales (*Physeter macrocephalus*) as an indicator species. J. Ecosyst. Ecogr. 1, 1. https://doi.org/10.4172/2157-7625.1000101.